



## Description and phylogenetic classification of *Stackelberginia cerberus* sp. nov. (Diptera: Asilidae), comprising the first record of this genus from the Nearctic

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### Abstract

*Stackelberginia cerberus* sp. nov. (Diptera: Asilidae) is described from the Amargosa desert (USA: Nevada) and compared to related taxa. This is the first record of the genus in the Western Hemisphere; other species live in the deserts of central Asia. *Stackelberginia* Lehr is proposed as the sister taxon to *Lasiopogon* Loew in the subfamily Stichopogoninae based on morphological characters and a Bayesian species tree estimated from one mitochondrial (*COI*) and three nuclear protein-coding loci (*AATS*, *PEPCK*, *wingless*). *Stackelberginia* has the medially divided epandrium and rotated hypopygium of *Lasiopogon*, but the facial gibbosity is flat, macrosetae of thorax, head, and legs are unusually long, and phenology peaks in late autumn.

**Key words:** Stichopogoninae, robber fly, assassin fly, species tree, molecular, Palearctic

### Introduction

Robber flies, also known as assassin flies (Diptera: Asilidae), are a major modern radiation of predators with more than 7500 species and 556 genera described and representatives in almost every terrestrial ecosystem (Pape *et al.* 2011, Dikow 2017). Reliable systematic treatments are essential for downstream analyses of ecology and evolutionary biology; however, most asilid taxa still await modern revisionary work. Diversity patterns in the subfamily Stichopogoninae have suggested repeated bouts of specialization and morphological and ecological character displacement (Lehr 1984), unfortunately, further exploration of these ideas has been hampered by our limited knowledge of phylogenetic relationships in this group.

While preparing forthcoming phylogenetic revisions for the genus *Lasiopogon* Loew 1847 and other Stichopogoninae, we discovered an undescribed species with unusual morphology from the Amargosa desert of southwestern Nevada. Classifying this new species provided an opportunity to revise our understanding of the long-overlooked genus *Stackelberginia* Lehr 1964 and consider its place in the Stichopogoninae lineage.

### Material and methods

Specimens were obtained from the personal collections of Eric Fisher and the first author and this material has been deposited in the following collections: Eric Fisher private collection, El Dorado Hills, CA, USA (FISH); National Museum of Natural History, Washington D.C., USA (USNM); Royal BC Museum, Victoria, BC, Canada (RBCM); University of Michigan Museum of Zoology, Ann Arbor, MI, USA (UMMZ). Specimens of *Lasiopogon canus* Cole and Wilcox 1938 and *L. schizopygus* Cannings 2002 from the RBCM collection were photographed to show alternate forms of the metacoxa. Type specimens of *Stackelberginia gracilis* Lehr 1964 and *S. tsharykulievi* Lehr 1964 were photographed by associates at the Russian Zoological Institute of Science, St. Petersburg, Russia (ZIN). Relevant passages from the original Russian literature were translated by the first author and are available on request.

**TABLE 1.** Taxonomy, collection data, genetic sampling, and voucher location for specimens used in the genetic analysis.

Taxon	Locality	GenBank #				Specimen Voucher #
		AATS	COI	PEPCK	WG	
<i>Cyrtopogon montanus</i> Loew 1874	USA: Oregon: Clackamas Co., Mt. Hood, Timberline lodge area; N 45.3326° W 121.7056°, 15 Jul 2014, col. T. & K. McKnight.	KY906309	KY914526	KY914509	-	UMMZ-TAM-692-1
<i>Dioctria hyalipennis</i> (Fabricius) 1794	USA: Michigan: Washtenaw Co., Ann Arbor, Bird Hills Nature Area, forest trail; N 42.3081° W 83.7600°, 31 May 2012; col. T. McKnight.	KY906300	KY914516	KY914499	-	UMMZ-TAM-347-1
<i>Lasiopogon cinctus</i> (Fabricius) 1781	Sweden: SÖ: Nyköping Strandbadet Byggninge, path to beach near parking; N 58.7801° E 17.3917°, 30 May 2009; col. K.C. Holston.	KY906298	KY914514	KY914497	KY914530	UMMZ-TAM-RBCM-17
<i>Lasiopogon fumipennis</i> Melander 1923	USA: Oregon: Hood River Co., Sherwood cmpgd, 6 mi E of Mt. Hood peak; N 45.3943° W 121.5698°, 15 Jun 2013; col. T. McKnight.	KY906304	KY914521	KY914504	KY914536	UMMZ-TAM-472-3
<i>Lasiopogon pacificus</i> Cole & Wilcox 1938	USA: Oregon: Lane Co., Sutton Creek cmpgd, forest trail to Alder dunes, 6 mi N of Florence; N 44.0555° W 124.1060°, 21 Jun 2013; col. T. McKnight.	KY906305	KY914522	KY914505	KY914537	UMMZ-TAM-488-1
<i>Lasiopogon slossonae</i> Cole & Wilcox 1938	USA: New York: Essex Co., W Branch Ausable River at 118 River Rd; N 44.2659° W 73.9590°, 2 Jun 2014; col. K. McKnight.	KY906297	KY914513	KY914496	KY914529	UMMZ-TAM-KBM2014060202
<i>Lasiopogon terricola</i> (Johnson) 1900	USA: Michigan: Washtenaw Co., sandy blowout in oak forest SW of Pickrel Lake; N 42.4120° W 83.9882°, 10 May 2013; col. T. McKnight.	KY906303	KY914520	KY914503	KY914535	UMMZ-TAM-404-1
<i>Lasiopogon willametti</i> Cole & Wilcox 1938	USA: Oregon: Marion Co., Willamette River sandbars at Willamette Mission SP, 4 mi W of Waconda; N 45.0821° W 123.0618°, 18 May 2012; col. T. & K. McKnight.	KY906299	KY914515	KY914498	KY914531	UMMZ-TAM-338-8
<i>Mydas clavatus</i> (Drury) 1773	USA: North Carolina: Wake Co., 4.8 km E Angier; N 35.5167° W 78.6667°, 5 Jul 2003; col. D. Pritchard.	EF650269	KT733514	-	-	USNMMENT00914563
<i>Ommatius tibialis</i> Say 1823	USA: Rhode Island, Washington Co., South Kingstown, Great Swamp; N 41.4611° W 71.5864°, 16 Jul 2006; col. T. Dikow, M. Thomas, K. Bayless.	EF650302	KT733263	-	-	USNMMENT00914193

.....continued on the next page

TABLE 1. (Continued)

Taxon	Locality	GenBank #				Specimen Voucher #	
		AATS	COI	PEPCK	WG		
<i>Ommatius tibialis</i> Say 1823	USA: Michigan: Oakland Co., Kensington Metropark, Martindale Dr. glade; N 42.5403° W 83.6295°; 14 Jul 2012; col. T. McKnight.	-	KY914519	KY914502	KY914534	UMMZ-TAM-379b-2	
<i>Proctacanthus hinei</i> Bromley 1928	USA: Michigan: Berrien Co., Warren Dunes SP; N 41.9123° W 86.6014°; 14 Jul 2012; col. T. McKnight.	KY906301	KY914517	KY914500	KY914532	UMMZ-TAM-360-1	
<i>Stackelberginia cerberus</i> <b>sp. nov.</b>	USA: Nevada: Clark Co., Wheeler wash, 6 mi NE of Pahrump; N 36.2468° W 115.8943°; 5 Oct 2013; col. T. McKnight.	KY906307	KY914524	KY914507	KY914539	UMMZ-TAM-509-1	
<i>Stackelberginia cerberus</i> <b>sp. nov.</b>	USA: Nevada: Nye Co., Rock Valley wash N of jet Hwy 95, 5 mi E of Amargosa Valley city; N 36.6336° W 116.3102°; 6 Oct 2013; col. T. McKnight.	KY906308	KY914525	KY914508	KY914540	UMMZ-TAM-512-1	
<i>Stichopogon argenteus</i> (Say) 1823	USA: Michigan: Leelanau Co., dunes W of D.H. Day group campgd; N 44.8949° W 86.0472°; 17 Aug 2013; col. T. McKnight.	KY906306	KY914523	KY914506	KY914538	UMMZ-TAM-502-1	
<i>Stichopogon barbistrellus</i> Loew 1854	Mongolia: Bayan-Olgii/Khovd Aimag: Bulgan Soum, stream confluence of Deed Nariin Gol with Bulgan Gol ~55 km N Bulgan; N 46.5531° E 91.3885°; 10 Jul 2009; col. Mongolian Aquatic Insect Survey, C.R. Nelson.	KY906296	KY914512	KY914495	-	UMMZ-TAM-CRN-9251-1	
<i>Stichopogon catulus</i> Osten Sacken 1887	USA: New Mexico: Grant Co., Gila River below Grapevine campsite; N 33.1779° W 108.2015°; 6 May 2015; col. T. & K. McKnight.	KY906310	KY914527	KY914510	-	UMMZ-TAM-754-1	
<i>Stichopogon punctum</i> Loew 1851	South Africa: KwaZulu-Natal; Cumberland Nature Reserve; S 29.5069° E 30.5064°; 13 Jan 2004; col. J. Londt, T. Dikow.	EF650315	KT733193	-	-	USNMMENT00914067	
<i>Stichopogon trifasciatus</i> (Say) 1823	USA: Michigan: Mason Co., Ludington SP, dunes S of Skyline dune; N 44.0318° W 86.4966°; 15 Jun 2012; col. T. McKnight.	KY906302	KY914518	KY914501	KY914533	UMMZ-TAM-366-2	
<i>Townsendia arenicola</i> Scarbrough 1995	USA: Florida: Polk Co., Broussard Catfish Creek SP, site 8; N 27.9827° W 81.4947°; 24 Jun 2010; col. M. & N. Deyrup, J. Dunlap.	-	KY914511	KY914494	KY914528	UMMZ-TAM-ARCH-2	

The known specimens of Asian *Stackelberginia* are more than 50 years old and beyond the capabilities of our molecular lab, so we only used specimens from the new American *Stackelberginia* to estimate its position in the species tree. To reduce computational complexity and uncertainty not essential for the intergeneric scope of this study, six *Lasiopogon* taxa were selected as representatives of the major species groups based on a phylogenetic revision of that genus currently in preparation. Species of *Stichopogon* Loew 1847 and *Townsendia* Williston 1895 with multiple nuclear loci sequenced were included to represent the other major branches of North American Stichopogoninae, and four asilids from other subfamilies and one mydid were included to serve as outgroups. Specimens used for genetic analysis are summarized in Table 1.

**Morphological processing.** Protocol for genitalia dissection, body measurements, and the species description format follow Cannings (2002), as does most descriptive morphological terminology. Antennae are described following Stuckenberg (1999), and wings are described following both McAlpine (1981) and Wootton and Ennos (1989). For compatibility with Cannings (2002), the following abbreviations are used for measurements: HW= head width, FW= face width, VW= vertex width, VD= vertex depth, GH= gibbosity height, GL= gibbosity length, LPP= length of postpedicel, WPP= width of postpedicel, LAS= length of antennal stylus, DCI= length from r-m crossvein to base of discal cell divided by overall length of discal cell (LPP, WPP, and LAS are respectively equivalent to LF1, WF1, and LF2+3 in Cannings (2002)). All specimens were measured for body length; two males and two females were used for other measurements. Images were taken with the Image-Pro Plus software via a Leica DC 300 camera mounted on a Leica MZ 16 dissecting microscope and measurements made in Adobe Photoshop CS5 12.0.4 after calibration with a stage micrometer.

Labels for primary types are copied verbatim following the system described by O'Hara (1982): listed from the top label downward, with data from each label enclosed in quotation marks and the lines of each label delimited by oblique slash marks. Information not found on the labels is given in square brackets. Other specimen records are not presented verbatim; all relevant locality information is included but localities have been edited for consistency and are listed alphabetically by country, state, and county.

**Molecular processing.** Specimens for molecular work were stored in 95% ethanol promptly after capture. At least the head, terminalia, wings, and one set of fore/mid/hind limbs were preserved as morphological vouchers for DNA-extracted specimens; these have been deposited at the UMMZ. Genomic DNA was extracted using Qiagen DNeasy Blood and Tissue or QIAamp DNA Micro kits respectively for larger or smaller specimens (QIAGEN Inc., Valencia, CA, USA), following the manufacturer's protocols; extractions were eluted into H<sub>2</sub>O and stored at -20°C.

Loci from one mitochondrial and three nuclear protein coding genes were amplified: cytochrome oxidase I (*COI*; 658 bp), phosphoenolpyruvate carboxykinase (*PEPCK*; 585 bp), wingless (*wg*; 553 bp), and alanyl-tRNA synthetase (*AATS*; 550 bp); primers for the PCR reactions are presented in Table 2. PCR amplifications were performed on an Eppendorf eppgradient Mastercycler using this universal recipe: 1 µL DNA template, 5.69 µL H<sub>2</sub>O, 1 µL 10x PCR buffer, 0.5 µL dNTPs, 0.2 µL BSA, 0.4 µL each primer, 0.75 µL 50 mM MgCl<sub>2</sub>, 0.06 µL Platinum Taq. Thermocycler protocols were as follows: *COI*: 35 cycles of 30 sec at 94°C, 30 sec at 46°C, 30 sec at 72°C; *wg*: 35 cycles of 45 sec at 94°C, 45 sec at 58°C (first 5 cycles touchdown from 64–60°C), 1 min at 72°C; *PEPCK* and *AATS*: 35 cycles of 45 sec at 94°C, 45 sec at 50°C (first 5 cycles touchdown from 56–52°C), 1 min at 72°C. Cycle steps for all samples were preceded by a denaturing step of 5 min at 94°C and followed by an extension step of 7 min at 72°C. Amplification success was verified by visualization on a 2% agarose gel; products were then purified with Affymetrix ExoSAP according to manufacturer's protocols and sequenced by the University of Michigan Sequencing Core on an Applied Biosystems 3730xl DNA Analyzer. All sequence files were visually inspected and cleaned in Sequencher 4.2 (GeneCodes Corp., Ann Arbor, MI, USA) and aligned in MEGA6 (Tamura *et al.* 2013) or MESQUITE 3.02 (Maddison and Maddison 2016). The *wg* alignment contained one variable-length intron that was removed before analysis, a short variable repeat section at amino acid (AA) positions 42–58, and a single AA indel at position 89; the other loci lack indels and were trivial to align.

A species tree was estimated using the random local clock species tree template in BEAST 2.4.3 (i.e., STARBEAST; Bouckaert *et al.* (2014), Drummond & Suchard (2010)), with *Mydas clavatus* (Drury 1773) (Mydidae) set as the outgroup. Each locus was partitioned independently during phylogenetic analysis; the third codon position in *COI* was unlinked from (1+2) to allow independent site and clock models but linked tree models with 0.5 ploidy (versus 2.0 ploidy for the nuclear loci). Optimal nucleotide substitution models compatible with the STARBEAST software for each locus and partition were determined following AIC and BIC in jModelTest 2.1.5 (Darriba *et al.* 2012, Guindon & Gascuel 2003): *AATS*, *PEPCK*, *COI* (1+2): GTR+G; *wg*: TrN+G; *COI* (3):

HKY+G; in all cases gamma was estimated with 4 categories. Default settings were kept for most parameters and priors, except setting the birth death tree model and constant populations. Two chains were run with length set to 200 million and sampling every 10 thousand. Parameter distributions and convergence of MCMC runs were assessed using Tracer 1.6 (Rambaut *et al.* 2014); chains were combined using LogCombiner, discarding the first 10% of each run as burn-in and resampling half as often (ESS for all priors was >200). Trees were visualized in FigTree 1.4 (Rambaut 2012).

**TABLE 2.** Primers used for genetic sequencing in this study.

Locus	Primer name	Primer sequence	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> (1994)
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> (1994)
Wingless	WG550F	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	Wild & Maddison (2008)
Wingless	WGABRZ	CACTTNACYTCRCARCACCARTG	Wild & Maddison (2008)
PEPCK	PK282F	GAAGGATGGCTBGCNGARCAYATG	Wild & Maddison (2008)
PEPCK	PK485R	GCAGCVGTNGCYTCRCTYCTCAT	Wild & Maddison (2008)
AATS	AATS1F40	GNATGAAYCARTTYAARCCNAT	Dikow (2009b)
AATS	AATS1R244	CATNCCRCARTCNATRTGYTT	Dikow (2009b)

## Taxonomy

### *Stackelberginia cerberus* sp. nov. McKnight

**Diagnosis.** A small grey species similar to *Lasiopogon* but with facial swelling mostly flattened; mystax confined to lower facial swelling; bristles of ocellar triangle, thorax, and legs extremely long and strong; occipital setae strong and straight; tarsal claws unusually large; metacoxa with anterior peg blunt; cuticle dark brown or black except for ferruginous tibiae and tarsi, mostly covered in grey tomentum; genitalia ferruginous, hypopygium rotated 120° and epandrium completely divided medially, narrow (Figs 1, 2).

**Description.** *Body length* ♂ 8.5–10 mm; ♀ 8.5–11.5 mm.

*Head.* Cuticle black; tomentum of face silver, vertex and occiput grey. Facial swelling greatly reduced, leaving only a slightly swollen angle above oral margin but otherwise not extending beyond eyes in lateral view. All setae on head white except ocellar and orbital bristles, which are typically chestnut brown. Mystax setae long, loosely clumped over oral margin with a small gap dorsomedially; frons with a clump of short, thick setae midway between antennae and ocellar triangle; one long, strong orbital seta anteriorly inclined, occasionally with another short fine seta; ocellar triangle with two long, strong setae and several short fine hairs anteriorly inclined along posterior edge, longest bristle to 0.7mm; occipital bristles erect, relatively short and straight, in lateral view extending ventrally to midpoint of eye. Palps with fine white hairs along length; robust setae on postgena.

HW ♂ 1.48–1.88mm; ♀ 1.64–1.88mm. FW ♂ 0.28–0.34mm; ♀ 0.32–0.35mm. VW ♂ 0.66–0.73mm; ♀ 0.67–0.77mm. GL ♂ 0.25–0.28mm; ♀ 0.25–0.33mm. VW/HW = ♂ 0.39–0.45; ♀ 0.41. FW/VW = ♂ 0.42–0.46; ♀ 0.46–0.48. VD/VW = ♂ 0.10–0.14; ♀ 0.12–0.13. GH/GL = ♂ 0.43–0.52 ♀ 0.42–0.48.

*Antennae.* Dark brown, base of pedicel pale brown. Robust white setae on scape and pedicel. Postpedicel rectangular with a slight bulge at midlength; stylus long, with pronounced apical spine. WPP/LPP = ♂ 0.22; ♀ 0.20–0.21. LAS/LPP = ♂ 0.50; ♀ 0.67–0.70.

*Thorax.* Thoracic cuticle dark brown, covered in thick grey tomentum. Prothorax with hairs white; postpronotal lobes chestnut brown with lateral angle reddish, hairs white; postalar lobes reddish. Scutum tomentum grey, without stripes or spots; short hairs sparse, white around perimeter and brown/black over dorsum. Dorsocentral bristles brown, prominent (longest to 0.85mm), 1 anterior and 2 posterior to transverse suture. Lateral bristles of the scutum brown, very long (longest to 1.3 mm): one postalar, one supra-alar, and two notopleural (one each of the presutural and posthumeral subgroups of Cannings 2002). Scutellum covered in grey tomentum, without impressed rim, bare on disc, 5–6 long white bristles apically with a few short fine hairs just inside.



Pleural tomentum grey, all hairs and bristles white. Katatergal setae 6–7 with a few finer hairs mixed dorsally; katepisternal setae fine, short; anepisternum with 2 long, prominent setae (longest 1.2mm) at posterior edge and a patch of fine hairs along dorsal margin; anepimeron without setae. Antepronotum with 8–12 fine setae; proepisternum with 4–6 strong long setae and 0–2 fine hairs.

*Legs.* Cuticle black except reddish tibia, trochanters, extreme medial and apical tips of femorae, and tarsal bases; tomentum grey, very thin below femora. Anterior face of metacoxa bear pronounced peg with apex rounded, not tapered; 2 fine setae projecting ventrally at base of peg. All hairs and bristles of legs white except on tarsi, where mostly brown. Coxae with dense, long, strong bristles facing anteriorly (on procoxa) or laterally (on meso- and metacoxa); 40+ on procoxa, 3–12 on mesocoxa, 4 on metacoxa. Femora with fine, short procumbent hairs and strong erect bristles; 5–6 ventral bristles longer than adjacent hairs on pro- and mesofemur, metafemur with only fine hairs just longer than surrounding setae. Apical and dorsolateral bristles strong, numerous: profemur with 3–5, mesofemur with 2–4, metafemur with 5–7. Bristles on tibiae and tarsi strong, long; protibia with longest bristle 3.5x longer than tibial width. Tarsal claws large, with reddish base and black apices.



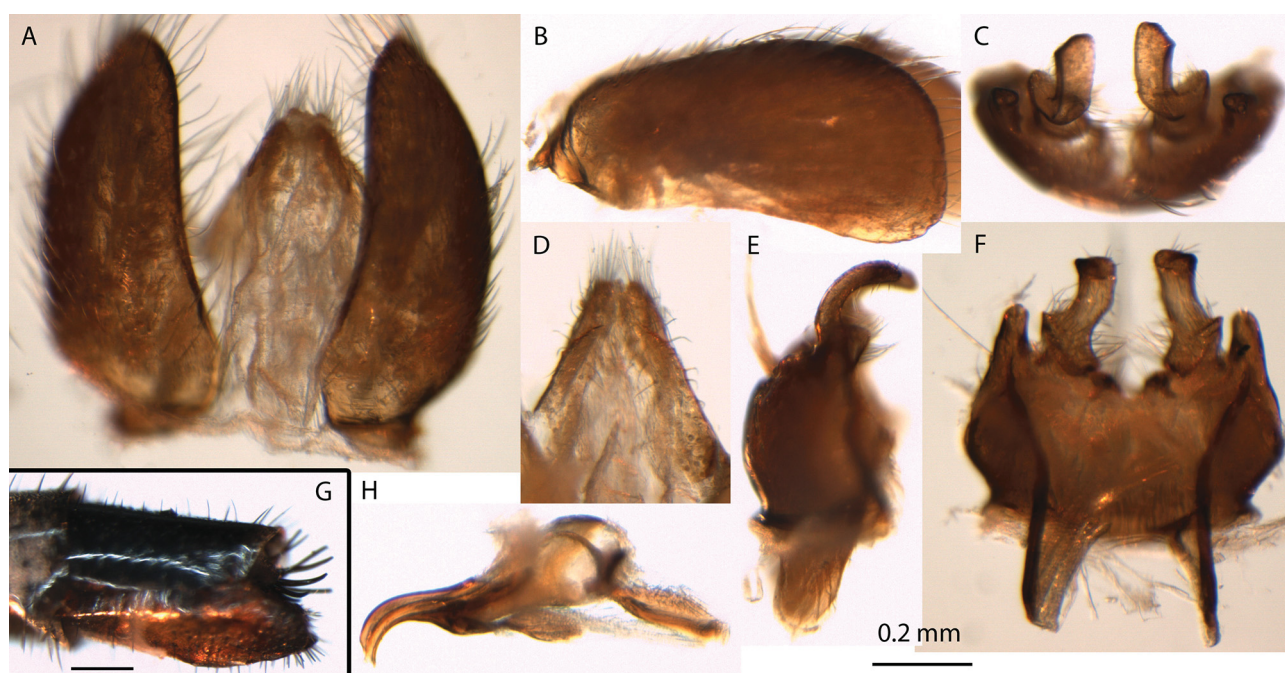
**FIGURE 1.** *Stackelberginia cerberus* sp. nov. female habitus and head detail. The male differs only in the terminalia, which are presented in Fig 2.

*Wings.* Veins yellow to dark brown, darker apically; membrane hyaline, slightly brown in oblique view. DCI = 0.52–0.58; cell m3 broadly open; cell cup (sensu McAlpine 1981; cua sensu Wootton & Ennos 1989) just closed at wing margin, varying from slightly stalked to slightly open in different individuals. Halter cream to light brown; knob without dark spot.

*Abdomen.* Tergite base color dark brown, light brown apically; covered in pale grey tomentum without other patterning. Tergite 1 with 6–8 strong bristles basolaterally, other tergites with strong short lateral setae, longest apically. Lateral setae white, dorsal setulae white laterally, black near midline. Sternite tomentum grey; setae white; sternite 1 with clump of 5–8 prominent erect setae. Tergite 8 red, visible between tergite 7 and terminalia. Terminalia red, with long hairs (white in male, black in female), in male rotated ~120° to one side.

*Male genitalia.* Epandrium and gonocoxite-hypandrial complex reddish-orange with darker apices, without tomentum, clothed in long white setae. Epandrium completely divided medially, in lateral view each half with width about 48% the length, widest at apex, ventral and dorsal margins mostly parallel, slumping ventrally, apex

truncate with rounded corners. In dorsal view, medial margins of epandrial halves very shallowly concave, almost straight. Epandrial apodeme shallow; basal sclerite absent (Cannings 2002).



**FIGURE 2.** *Stackelberginia cerberus* sp. nov. genitalia detail: A) epandrium, dorsal; B) epandrium lateral; C) gonostylus, apical; D) subepandrial sclerite, ventral; E) gonocoxite + hypandrium complex, ventral; F) gonocoxite + hypandrium complex, lateral; G) female ovipositor, lateral; H) phallus, lateral. Scale bars are 0.2 mm.

Gonostylus elongate, slender, curved dorsally like a hooked finger, with another point dorsolaterally at the base. Long hairs over basal part of gonostylus. Gonocoxite-hypandrial complex in ventral view with width about 86% the length, partially fused laterally, medial transverse slit semicircular with distally pointed apex. Medial setal brush on gonocoxite-hypandrial complex white, setae long but relatively sparse compared to *Lasiopogon*. Gonocoxal apodemes long, in lateral view exposed length about 70% the basal width of the hypandrium; apodeme with sclerotized web ventrally.

Phallus paramere sheath long and slender, curved ventrally like a goose neck. Dorsal carina a low ridge no wider than the paramere sheath itself that follows the curvature of the paramere sheath and terminates before the apex without a notch. Subepandrial sclerite V-shaped; triangular medial unsclerotized portion in basal 65%; spines slender, attenuate, sparsely scattered over surface.

**Female genitalia.** (Undissected) Hairs on segment 8 dark brown/black, erect, abundant. Tergite 8 black, sternite 8 red/yellow, hypogynial valves dark brown, with many dark hairs; lateral lobe with dark setae; acanthophorite spines black, 3 on each half.

**Type Material.** HOLOTYPE. ♂ labelled: "[rectangular white label] NEV: Nye Co: NTS [Nuclear Test Site]/ Rock Valley/ IX-12-76, can tr./E.L. Sleeper et al."; "[rectangular white label] ?/ det. EMFisher"; "[rectangular white label] FISH"; "[rectangular white label] mirus". A holotype label "HOLOTYPE/ *Stackelberginia/ cerberus/* des. T.A. McKnight 2017 [red, black-bordered label]" has been attached to this specimen. USNM type #USNM01295437. Both antennae are missing the postpedicel.

PARATYPES (8 designated, including 3 with genomic DNA extracts). **U.S.A.: Nevada**, Clark Co: Wheeler wash 6 mi NE of Pahrump, N 36.24678° W 115.89428°, elev: 1251 m, 5.x.2013, T.A. McKnight (1♀ [DNA voucher] UMMZ-TAM-509-1, 1♀ [DNA voucher] UMMZ-TAM-509-2; 1♀ [EtOH] UMMZ-TAM-509-3; 1? [damaged by dermestids] UMMZ-TAM-509-4); Nye Co: NTS [Nuclear Test Site] Rock Valley can tr., 3.x.1975, E.L. Sleeper (1♀ [dissected] RBCM ENT017-001946), 12.ix.1976, E.L. Sleeper (1♂ [dissected] RBCM ENT017-001945); Rock Valley wash jct Hwy 95, 5 mi E of Amargosa Valley city, N of hwy, N 36.63359° W 116.31018° elev: 878 m, 6.x.2013, T.A. McKnight (1♀ [DNA voucher] UMMZ-TAM-512-1; 1♀ FISH).

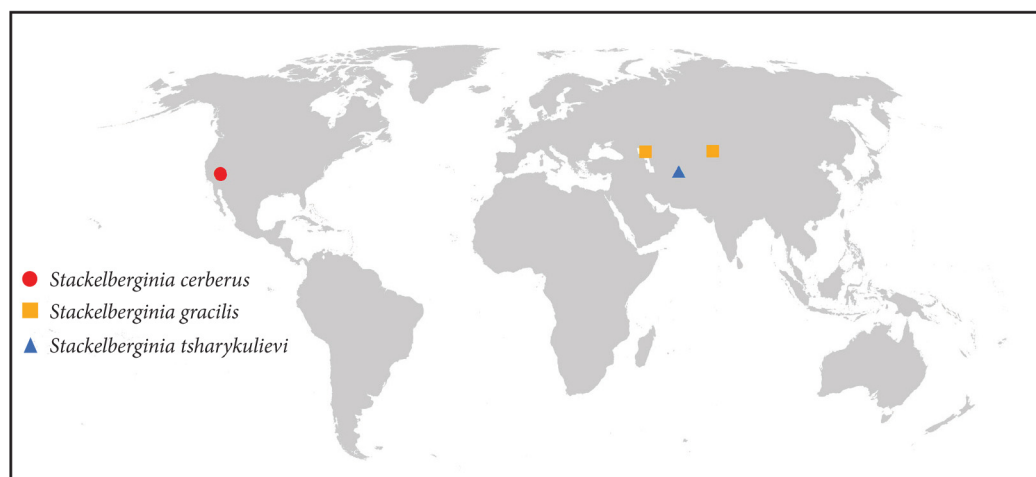
**Type Locality.** U.S.A.; Nevada, Nye Co., Nuclear Test Site, Rock Valley [N 36.694° W 116.179°].



**Taxonomic Notes.** This species was included in Cannings (2002) as “*L. unc-7 sp. nov.*” and had been assigned a manuscript name of “*mirus*” because it was thought to be a miraculously unusual *Lasiopogon*. This epithet was changed after reclassification outside *Lasiopogon*.

**Etymology.** Latin *Cerberus* from the Greek Κέρβερος, used as a noun in apposition, named in similarity to the hound guarding the gates of the underworld in Greek mythology. The type locality for this predator is just outside the boundaries of both Death Valley National Park (the lowest elevation in North America) and the Yucca Mountain nuclear waste facility—sites with a suitably Hadean flair.

**Distribution.** Nearctic: USA; known only from two sites in the Amargosa desert of southwestern Nevada. Previously described species of *Stackelberginia* are known from deserts in Kazakhstan and Turkmenistan (Fig. 3); we have summarized localities from the literature (Lehr 1964, 1980) below with estimated georeferenced coordinates:



**FIGURE 3.** *Stackelberginia* world distribution.

*S. gracilis*: Kazakhstan: Almaty Oblast: River Ila at Ayakkalkan resort, sandy crest among gravel, claypan, and marshy hollows on left bank [N 43.861° E 78.424°], 7.ix-9.x.1952 (number of specimens not noted); Mangishlyak Peninsula: Baskuduk district, salt flats and claypan amidst marshy hollows and dune chains [N 43.696° E 51.227°], 7.ix.1960, col. P.A. Lehr (1♂).

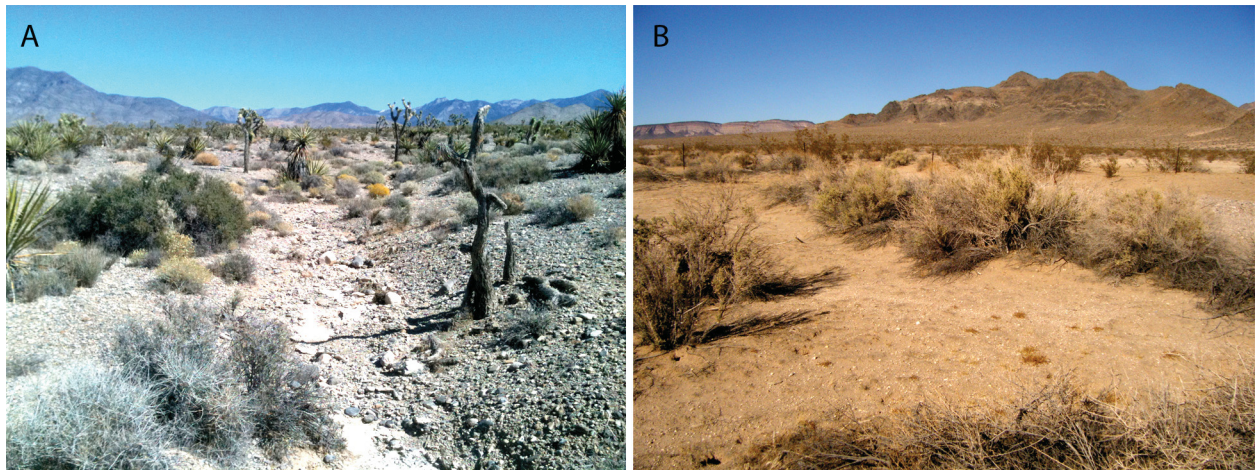
*S. tsharykulievi*: Turkmenistan: Mary: Leskhoz, desert region, on sand [N 37.291° E 62.351°], 9.x.1959, col. D.M. Charykulievi (1♂ 2♀).

**Natural History.** Habitat: sandy dry desert washes, usually found perching on fist-sized rocks in sandy areas near clumps of vegetation (Fig. 4). One female was observed preying on a slightly-smaller fly (not collected). The fall flight period (12 September–6 October) is distinctive within the subfamily.

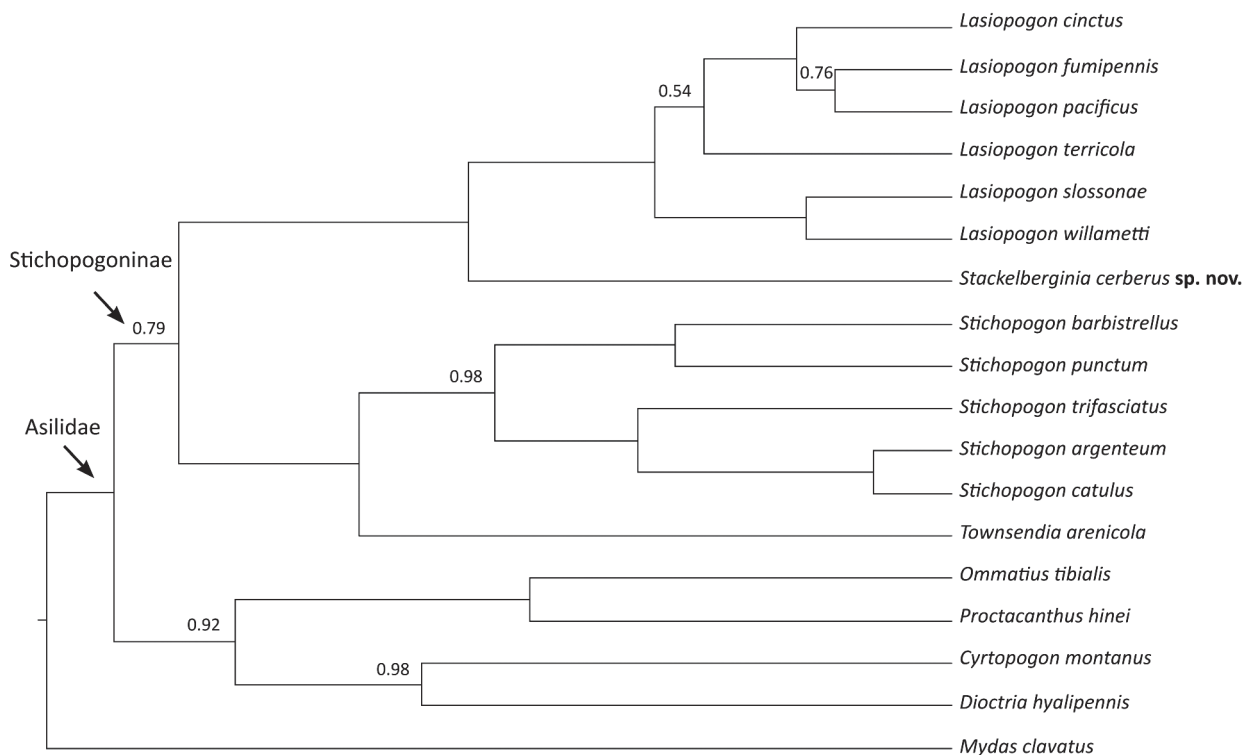
Other assassin flies were also active at the *Stackelberginia* sites at the same time. These were identified using keys in Wood (1981) and Wilcox (1961, 1965, 1966) and voucher specimens have been deposited at the RBCM and UMMZ: *Hodophylax basingeri* Pritchard, *Cophura fisheri* Wilcox, *Efferia* (*Aridefferia*) *basingeri* Wilcox.

**Phylogeny.** The species tree supports *Stackelberginia* as sister to *Lasiopogon*, and this clade as sister to the rest of the subfamily (Fig. 5); these relationships were also recovered in each of the four individual gene trees (not shown). Node posterior probabilities are reasonably high ( $\geq 0.92$ ) throughout the tree, except for some uncertainty for phylogenetic relationships within *Lasiopogon* and at the Stichopogoninae root. Lower support values for the subfamily root appear to stem from a basal polytomy in COI; this locus is known to have trouble resolving deeper level phylogenetic relationships (Matsuda *et al.* 2014). Intrageneric relationships within *Lasiopogon* and *Stichopogon* will be covered in future publications with more comprehensive taxon sampling, but the overall genus-level topology matches our prior expectations from morphology—e.g., *Townsendia* grouping with *Stichopogon*, and the Asilinae (*Proctacanthus* Macquart) and Ommatiinae (*Ommatius* Wiedemann) clustering together in the outgroup.





**FIGURE 4.** Habitats where *Stackelberginia cerberus* **sp. nov.** were collected in Nevada (see specimen list for more precise locality data): A) Clark Co.: Wheeler wash; B) Nye Co.: Rock Valley wash. Individuals were caught perching on small stones and sand along the washes.



**FIGURE 5.** Species tree for representatives of *Stackelberginia*, *Lasiopogon*, *Stichopogon*, *Townsendia*, and Asilidae and Mydidae outgroups, estimated via Bayesian inference in STARBEAST2 from one mitochondrial locus (*COI*) and three nuclear protein coding loci (*wingless*, *PEPCK*, *AATS*). Branch labels show support for nodes when posterior probability <1.

## Discussion

These specimens were initially thought to represent an extraordinary new *Lasiopogon* species (hence its inclusion in Cannings (2002) as “*L. unc-7*”) but comparison to the text and figures of *Stackelberginia* descriptions (Lehr 1964, 1984) and to photographs of type specimens at St. Petersburg (Fig. 6) suggest a closer relationship to *Stackelberginia*. Rationale for this taxonomic decision is enumerated below. However, because the most informative characters for resolving fine-scale relationships in this group depend on internal morphology that was unavailable in the texts and images of Palearctic *Stackelberginia*, it remains uncertain if these disparately

distributed taxa actually constitute a single monophyletic group or are merely similar but independent relic branches left from the early diversification of the Stichopogoninae lineage. We invite entomologists collecting in Central Asia to collaborate in the hunt for newer specimens that could be made available for genitalia dissection or DNA sequencing.



**FIGURE 6.** Holotypes of *Stackelberginia tsharykulievi* (A, B, E) and *S. gracilis* (C, D, F); lateral, dorsal and head detail.

Higher level relationships in the Asilidae have been repeatedly addressed over the last century, but *Stackelberginia* remained largely forgotten on the sidelines, likely because the genus is known from only a few specimens collected long ago in a region now difficult to access. Following Karl's (1959) cladistic insights on male genitalia in the Asilidae, Lehr (1984) recognized two separate branches of Stichopogoninae: one with unrotated terminalia and the epandrium fused into a single hood-like structure (as in *Stichopogon* and most of the group); the other with terminalia inverted and epandrium halves completely divided medially (e.g., *Lasiopogon* and *Stackelberginia*). This hypothesis was described verbally, not statistically tested, but it represents the only prior supposition and justification of *Stackelberginia* phylogeny. Morphological and molecular parsimony analyses in recent years (Cannings 2002, Dikow 2009a, 2009b) passed over *Stackelberginia* due to lack of specimens but otherwise supported a monophyletic subfamily with *Lasiopogon* as the sister taxon to the rest of the subfamily.

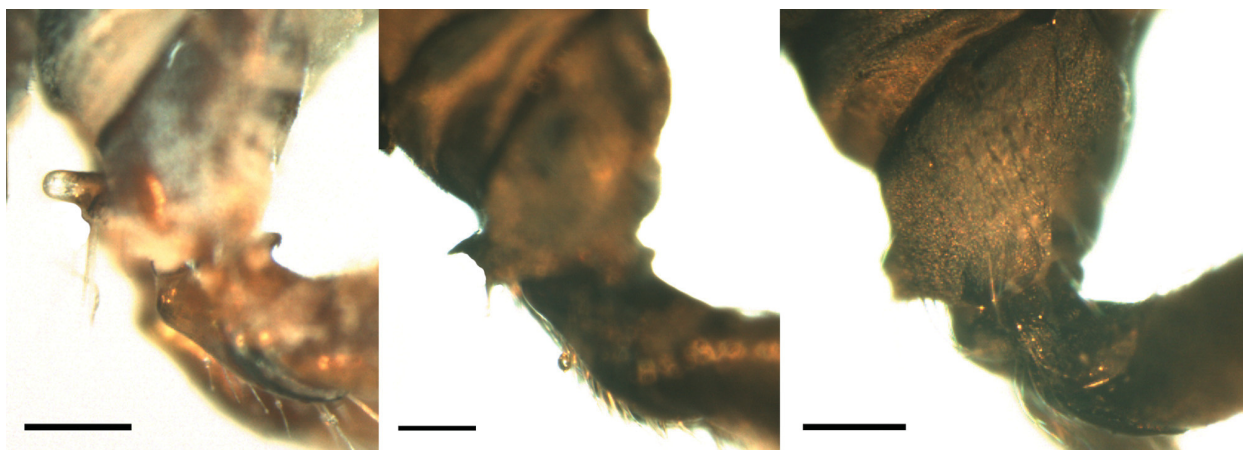
*Stackelberginia* clearly belongs in the Stichopogoninae, with the Nearctic and Palearctic species all bearing the primary defining characters of the subfamily: dorsally widening frons, sinuate lower posterior margin of the compound eye (albeit slightly), anterior anepisternal setae absent, and female sternite 8 platelike with flattened and separated hypogynial valves (Dikow 2009a, Cannings 2002). The state of the prosternum and proepisternum is not visible in our photographs of Central Asian *Stackelberginia*, but are fused in *S. cerberus* **sp. nov.** However, unlike other members of the subfamily (Dikow and Grimaldi 2014), the wing cell cup (sensu McAlpine 1981; *cua* sensu Wootton & Ennos 1989) is variably open or closed in different individuals of *S. cerberus* **sp. nov.**

The case for classifying this new species with the Central Asian *Stackelberginia* is based on morphological, phylogenetic, and ecological data. (i) Morphology: important characters shared by these taxa include: rotated male terminalia; medially divided epandrium; gonocoxite-hypandrium complex with medial setal brush; facial swelling mostly flattened; mystax confined to just above oral margin; postocular macrosetae mostly straight and present from vertex to level of occipital foramen; bristles of the mesonotum, mesopleuron, coxae, and legs unusually long and strong; two notopleural bristles; body covered in thick tomentum; and strong claws. Some of these characters (especially the male genitalia) are also found in *Lasiopogon*, but not in combination with the other traits. Notopleural bristles in *Lasiopogon* range from 1–6, but most taxa have 3–4. (ii) Phylogeny: as noted above, Lehr did not publish a cladogram that included *Stackelberginia*, but his text (Lehr 1984) indicates a belief that the genus



is properly grouped with *Lasiopogon* and not with *Stichopogon*, *Townsendia*, or others. Our molecular phylogeny strongly supports the placement of *S. cerberus* **sp. nov.** as sister to all Nearctic and Palearctic *Lasiopogon*, but with a substantial branch length between the genera, a topology consistent with Lehr's suggestion. (iii) Ecology: although little is known of the ecology of these species, they are notably different from *Lasiopogon* by emerging during the autumn (Sept–Oct) instead of the spring or summer. Habitats for *Stackelberginia* from both continents (Lehr 1964, 1980, 1984, 1988) can be characterized as desert areas where sandy and gravelly patches mix near intermittent water (e.g., dry washes, alkali flats); they are found perching directly on the sand or on nearby rocks or plants.

Despite these shared characters, there are some differences, most notably in the morphology of the antenna and the coxal pegs. First, the postpedicel of *S. tsharykulievi* is longer and narrower than in *S. cerberus* **sp. nov.** and apparently has the stylus short and slightly inset apically; the postpedicel and stylus of the *S. gracilis* holotype are missing. Second, neither of the Central Asian species appears to have a metacoxal peg, though the pictures do not definitively show this area. However, the stout rounded metacoxal peg of *S. cerberus* **sp. nov.** is unlike any found in *Lasiopogon* (which are sharp and short if present, Fig 7) and instead resembles the pegs found in some other groups of Stichopogoninae (e.g., some *Stichopogon*, some *Lissoteles* Bezzi 1910, *Rhadinus* Loew 1856), and scattered in select taxa of other asilid subfamilies, including Bathypogoninae, Brachyrhopalinae, Dasypogoninae, Stenopogoninae, Tillobromatinae, Willistoninae, and the incertae sedis *Coleomyia* (character states summarized from Dikow 2009a and personal observations). Similar blunt methathoracic pegs are found in several other families throughout the Asiloidea, including Apioceridae, Apsilocephalidae, Bombyliidae, Scenopinidae, and Therevidae, and are thought to be plesiomorphic for the group (Winterton 2004).



**FIGURE 7.** Coxal peg types in Stichopogoninae: A) blunt, robust (*Stackelberginia cerberus* **sp. nov.**); B) sharp, short (*Lasiopogon canus*); C) no peg (*Lasiopogon schizopygus*). Scale bar is 0.2 mm.

In the commonly used key to genera from the Manual of Nearctic Diptera (Wood 1981), *S. cerberus* **sp. nov.** runs to couplet 22a (*Stichopogon*); it can be distinguished from *Stichopogon* by having two notopleural bristles (instead of one), occipital setae continuing ventrally to midpoint of eye when viewed laterally (instead of ending in the dorsal quarter), and the medially divided epandrium in the male (instead of a fused hoodlike sclerite).

While both *Lasiopogon* and *Stackelberginia* have Holarctic distributions, the three known *Stackelberginia* species are found in hot southern pockets of relatively low *Lasiopogon* diversity. There are approximately 68 species of *Lasiopogon* known from the Nearctic, and 64 from the Palearctic (Cannings (2002), with new species descriptions in prep added), but none are known to inhabit the Amargosa Valley, and only 2–3 species are found in nearby mountain ranges (Cannings (2002), with new species descriptions in prep added). Similarly, only one species of *Lasiopogon* is known from Kazakhstan or the former Soviet Middle Asia region despite the history of Lehr's extensive collecting activity in the area (Cannings 2002).

Coming on the heels of a recent review of southern Nevada robber flies that documented many new state records (Stevens & Scarbrough 2015); it is evident that undescribed fly diversity abounds even in the relatively well-sampled United States. The extremely patchy nature of robber fly distributions is undoubtedly both a blessing and a curse for biologists: it may help species diversify and allow flies to persist in fragmented habitats, but it also may prevent their discovery even by specialists. The sites where *S. cerberus* **sp. nov.** was collected fall just outside the main areas studied by Stevens and Scarbrough, i.e., Ash Meadows National Wildlife Refuge and Spring

Mountains National Recreation Area. It is possible that other undescribed species of *Stackelberginia* remain in the deserts of the American Southwest.

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## References

- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10 (4), e1003537.  
<https://doi.org/10.1371/journal.pcbi.1003537>
- Bromley, S.W. (1928) Notes on the genus *Proctacanthus* with the descriptions of two new species (Diptera: Asilidae). *Psyche*, 35, 12–15.  
<https://doi.org/10.1155/1928/48636>
- Cannings, R.A. (2002) *The systematics of Lasiopogon (Diptera: Asilidae)*. Royal British Columbia Museum, Victoria, 354 pp.
- Cole, F.R. & Wilcox, J. (1938) The genera *Lasiopogon* Loew and *Alexiopogon* Curran in North America (Diptera-Asilidae). *Entomologica Americana*, 43, 1–91.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9 (8), 772.  
<https://doi.org/10.1038/nmeth.2109>
- Dikow, T. (2009a) Phylogeny of Asilidae inferred from morphological characters of imagines (Insecta: Diptera: Brachycera: Asiloidea). *Bulletin of the American Museum of Natural History*, 319, 1–174.  
<https://doi.org/10.1206/603.1>
- Dikow, T. (2009b) A phylogenetic hypothesis for Asilidae based on a total evidence analysis of morphological and DNA sequence data (Insecta: Diptera: Brachycera: Asiloidea). *Organisms Diversity & Evolution*, 9, 165–188.  
<https://doi.org/10.1016/j.ode.2009.02.004>
- Dikow, T. (2017) Asiloid Flies: Asilidae generic classification sensu Dikow 2009. Available from: <https://zenodo.org/record/14704#.WX1CAtSGOUk> (accessed 23 May 2017)
- Dikow, T. & Grimaldi, D.A. (2014) Robber flies in Cretaceous ambers (Insecta: Diptera: Asilidae). *American Museum Novitates*, 3799, 1–19.  
<https://doi.org/10.1206/3799.1>
- Drummond, A.J. & Suchard, M.A. (2010) Bayesian random local clocks, or one rate to rule them all. *BMC Biology*, 8, 114.  
<https://doi.org/10.1186/1741-7007-8-114>
- Drury, D. (1773) *Illustrations of natural history. Vol. 2*. B. White, London, 92 pp.  
<https://doi.org/10.5962/bhl.title.61910>
- Fabricius, J.C. (1781) *Species insectorum exhibentes eorum differentias specificas, synonyma, avctorum, loca natalia, metamorphosin adiectis observationibus, descriptionibus. Tome II*. C.E. Bohn, Hamburg & Kiel, 517 pp.  
<https://doi.org/10.5962/bhl.title.36509>
- Fabricius, J.C. (1794) *Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species adiectis synonymis, locis, observationibus, descriptionibus. Tome IV*. C.G. Proft, Copenhagen, 477 pp.  
<https://doi.org/10.5962/bhl.title.36532>
- Folmer, I., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, 52, 696–704.  
<https://doi.org/10.1080/10635150390235520>
- Johnson, C.W. (1900) Notes and descriptions of seven new species and one new genus of Diptera. *Entomological News*, 11, 323–328.
- Karl, E. (1959) Vergleichend-morphologische Untersuchungen der männlichen Kopulationsorgane bei Asiliden (Diptera). *Beiträge Entomologie*, 9, 619–680. [in German]
- Lehr, P.A. (1964) [New genera and new species of the robber-flies (Diptera, Asilidae) in the fauna of the USSR]. *Entomologicheskoe Obozrenie*, 18 (4), 914–934. [in Russian]



- Lehr, P.A. (1980) [Eurybiotic mode of life as the initial stage of evolution of a taxon, by the example of robber flies (Diptera, Asilidae)]. In: [Parallelism and Regularity in the Evolution of Insects] Far East Division of the Academy of Sciences of the USSR, Vladivostok, pp. 4–69. [in Russian]
- Lehr, P.A. (1984) [Assassin flies of the tribe Stichopogonini (Diptera, Asilidae) of the fauna of the USSR. 2]. *Zoologicheskii Zhurnal*, 63 (6), 859–864. [in Russian]
- Lehr, P.A. (1988) Family Asilidae. In: Soós, Á. & Papp, L. (Eds.), *Catalogue of Palearctic Diptera, 5, Athericidae-Asilidae*. Akadémiai Kiadó, Budapest, pp. 197–326.
- Loew, H. (1851) Bemerkungen über die Familie der Asiliden. *Programm Königlichen Realschule zu Meseritz*, 1851, 1–22.
- Loew, H. (1854) Neue Beiträge zur Kenntnis der Dipteren. Zweiter Beitrag. *Programm Königlichen Realschule zu Meseritz*, 1854, 1–24.
- Loew, H. (1874) Neue nordamerikanische Dasypogonina. *Berliner Entomologische Zeitschrift*, 18, 353–377.  
<https://doi.org/10.1002/mmnd.18740180322>
- Maddison, W.P. & Maddison, D.R. (2016) Mesquite: a modular system for evolutionary analysis. Version 3.02. Available from: <http://mesquiteproject.org> (accessed 24 Mar 2015)
- Matsuda, T., Morishita, M., Hinomoto, N. & Gotoh, T. (2014) Phylogenetic Analysis of the Spider Mite Sub-Family Tetranychinae (Acari: Tetranychidae) Based on the Mitochondrial COI Gene and the 18S and the 5' End of the 28S rRNA Genes Indicates That Several Genera Are Polyphyletic. *PLoS ONE*, 9 (10), e108672.  
<https://doi.org/10.1371/journal.pone.0108672>
- McAlpine, J.F. (1981) 2. Morphology and terminology—adults. In: McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Coords.), *Manual of Nearctic Diptera. Vol. 1. Agriculture Canada, Ottawa*, pp. 9–63.
- Melander, A.L. (1923) The genus *Lasiopogon* (Diptera, Asilidae). *Psyche*, 30, 130–45.  
<https://doi.org/10.1155/1923/20592>
- O'Hara, J.E. (1982) Classification, phylogeny and zoogeography of the North American species of *Siphona* Meigen (Diptera: Tachinidae). *Quaestiones Entomologicae*, 18, 261–380.
- Osten Sacken, C.R. (1887) Diptera [part]. In: Godman, F.D. & Salvin, O. (Eds.), *Biologia Centrali- Americana. Zoologia. Insecta. Diptera. Vol. 1*. Taylor Francis, London, 378 pp.  
<https://doi.org/10.5962/bhl.title.730>
- Pape, T., Blagoderov, V.A. & Mostovski, M.B. (2011) “Order Diptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.) *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness*. *Zootaxa*, 3148, 222–229.
- Rambaut, A. (2012) Figtree v1.4. Available from: <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 8 June 2014)
- Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) Tracer v1.6. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 9 June 2014)
- Say, T. (1823) Descriptions of dipterous insects of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*, 3, 9–54, 73–104.
- Scarborough, A.G., Norden, B.B. & Krombein, K.V. (1995) A new species of *Townsendia* Williston (Diptera: Asilidae) from Florida with notes on its association with *Perdita graenicheri* Timberlake (Hymenoptera: Andrenidae). *Proceedings of the Entomological Society of Washington*, 97, 689–694.
- Stevens, L.E. & Scarborough, A.G. (2015) Additions to the robber fly fauna of southern Nevada, USA (Diptera: Asilidae). *Journal of the Arizona-Nevada Academy of Science*, 46 (2), 42–49.  
<https://doi.org/10.2181/036.046.0204>
- Stuckenberg, B.R. (1999) Antennal evolution in the Brachycera (Diptera), with a reassessment of terminology relating to the flagellum. *Studia Dipterologica*, 6, 33–48.  
<https://doi.org/10.5281/zenodo.12390>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.  
<https://doi.org/10.1093/molbev/mst197>
- Wilcox, J. (1961) The genus *Hodophylax* James (Diptera: Asilidae). *Bulletin of the Brooklyn Entomological Society*, 61, 112–116.
- Wilcox, J. (1965) New *Cophura*, with revised keys to the groups and to the Nearctic species (Diptera: Asilidae). *Annals of the Entomological Society of America*, 58 (6), 805–816.  
<https://doi.org/10.1093/aesa/58.6.805>
- Wilcox, J. (1966) *Efferia* Coquillett in America north of Mexico (Diptera: Asilidae). *Proceedings of the California Academy of Sciences*, 34 (2), 85–234.
- Wild, A.L. & Maddison, D.R. (2008) Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, 48, 877–891.  
<https://doi.org/10.1016/j.ympev.2008.05.023>
- Winterton, S.L. (2004) Are hind coxal knobs a synapomorphy for therevids?: An unusual new species of *Anabarhynchus* Macquart from Australia (Diptera: Therevidae: Therevinae). *Zootaxa*, 413 (1), 1–8.  
<https://doi.org/10.11646/zootaxa.413.1.1>
- Wood, G.C. (1981) Family Asilidae. In: McAlpine, J.R., Peterson, B.V., Shell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds.), *Manual of Nearctic Diptera. Vol. 1. Agriculture Canada, Hull*, pp. 549–573.
- Wootton, R.J. & Ennos, A.R. (1989) The implications of function on the origin and homologies of the dipterous wing. *Systematic Entomology*, 14, 507–520.  
<https://doi.org/10.1111/j.1365-3113.1989.tb00300.x>